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A PRELIMINARY SURVEY OF PHENOLIC COMPOUNDS IN THE GENUS *STREPTOCARPUS* (GESNERIACEAE)

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ABSTRACT

The phenolic compounds were investigated in the leaves of 55 accessions representing 27 species of *Streptocarpus*. A two-dimensional chromatographic technique was employed and a total of 22 compounds was separated out. Because of the relatively few compounds present, evaluation of the results was very difficult and no clear-cut answers to taxonomic problems in the genus emerged. The narrowness of the spectrum of species studied was also a limiting factor in interpreting the results.

UITTREKSEL

'N VOORLOPIGE ONDERSOEK VAN DIE FENOLIESE VERBINDINGS IN DIE GENUS *STREPTOCARPUS* (GESNERIACEAE). Die teenwoordigheid van fenoliese verbindings in die blare van 55 versamelings verteenwoordigend van 27 soorte van *Streptocarpus* is ondersoek. 'n Twee-dimensionele chromatografiese metode is vir die doel aangewend en 22 verbindings is van mekaar geskei. Die geringe aantal fenoliese verbindings teenwoordig, het die verwerking van die resultate bemoeilik. Gevolglik kon geen definitiewe gevolgtrekkings aangaande taksonomiese probleme in die genus gemaak word nie. Die feit dat slegs 27 soorte ontleed is, het ook hiertoe bygedra.

INTRODUCTION

Available information indicates that the chromatographic separation of phenolic compounds may provide information that is valuable in supplementing data from other sources for distinguishing species and determining species interrelationships. Phenolic compounds have been investigated in chemotaxonomic studies of a number of genera, including *Baptisia* (Alston & Turner, 1959, 1963), *Trifolium* (Collins & Taylor, 1964); *Lotus* (Harvey & Grant, 1964); *Eucalyptus* (Hillis & Isoi, 1965); *Psoralea* (Ockendon, Alston & Naifeh, 1965); and *Medicago* (Simon, 1967; Simon & Goodall, 1968).

In the genus *Baptisia*, it was demonstrated that phenolic compounds are more reliable indicators of species relationships than are alkaloids or amino acids (Brehm & Alston, 1964). Harborne (1966) studied the systematic distribution of 3-desoxyanthocyanins in the family Gesneriaceae. His findings supported Burtt's (1962) reclassification of the family into two sub-families on the basis of geographical distribution and the presence or absence of anisocotyly.

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TABLE 1

POLYPHENOLIC COMPOUNDS SEPARATED BY TWO-DIMENSIONAL CHROMATOGRAPHY IN *STREPTOCARPUS*

SPECIES	LOCALITY		ACCESSION NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
SUBGENUS STREPTOCARPUS																									
*_S. wendlandii_	Natal, Ngoye	28° 51′ S, 31° 41′ E	Hilliard 2707	×	×	×	×	·	×	·	×	×	×	·	×	·	·	·	·	×	·	·	·	·	·
S. molweniensis																									
*subsp. _molweniensis_	Natal, Everton	29° 48′ S, 30° 51′ E	No number	×	×	×	×	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·	·	·	·
*subsp. _nov._ (†H & B ined.)	Natal, Eshowe dist.	28° 52′ S, 31° 28′ E	H & B 5655	×	×	×	×	×	×	·	·	·	·	·	×	·	·	·	·	×	×	·	·	·	·
*_S. grandis_	Natal, Nkandla	28° 43′ S, 31° 9′ E		·	×	×	×	·	×	·	·	×	·	·	·	·	·	·	·	·	·	·	·	·	×
*_S. trabeculatus_	Natal, Port Shepstone dist.	30° 44′ S, 30° 28′ E	H & B 6062	·	×	×	×	·	×	·	×	×	·	·	×	×	·	·	·	·	·	·	·	×	·
*_S. pole-evansii_	Transvaal, Nelspruit dist.	25° 27′ S, 30° 58′ E	H & B 6039	·	×	×	×	·	×	·	×	×	·	·	·	·	·	·	·	·	·	×	·	·	·
S. dunnii	Transvaal, Barberton-Havelock Rd.	25° 57′ S, 31° 6′ E	H & B 3665	·	×	×	×	·	×	·	·	·	·	·	·	·	·	·	·	·	·	×	·	·	·
S. montanus	Teita Hills, Kenya		Burtt 3830	·	×	×	×	·	×	·	×	·	·	·	·	·	·	·	·	·	·	·	·	·	·
S. fanniniae	Natal, Richmond dist.	29° 52′ S, 30° 16′ E	Hilliard s.n.	·	×	×	×	·	×	·	·	×	·	·	×	×	·	·	·	·	·	·	·	·	·
	Natal, Lions River	29° 28′ S, 30° 9′ E	Hilliard s.n.																						
S. candidus	Natal, Ngotshe dist.	27° 50′ S, 31° 22′ E	Hilliard 863	·	×	×	×	·	×	×	×	×	·	·	·	·	·	×	·	·	·	·	·	·	·
	Natal, Mhlabatini dist.	28° 15′ S, 31° 28′ E	H & B 3296																						
	Natal, Vryheid dist.	28° 10′ S, 31° 11′ E	Hilliard 4785																						
*_S. wilmsii_	Transvaal, Pilgrims Rest dist.	24° 33′ S, 30° 53′ E	H & B 5974	·	×	×	×	·	×	·	·	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. kentaniensis	E. Cape, Kentani	32° 30′ S, 28° 19′ E	Strey 6651	·	×	×	×	·	×	·	·	×	·	·	×	·	·	·	·	·	·	·	×	·	·
S. modestus	E. Cape, Lusikisiki dist.	31° 28′ S, 29° 38′ E	Gillies s.n.	·	×	×	×	·	×	×	·	×	·	··	·	·	·	·	·	·	·	·	·	·	·
S. nov. (H & B ined.)	Transvaal, Zoutspansberg	23° 2′ S, 29° 25′ E	Hilliard 4760	·	×	×	×	·	×	·	·	×	·	·	×	·	·	·	·	·	·	·	·	·	·
S. rexii	E. Cape, Port Elizabeth dist.	33° 20′ S, 25° 45′ E	Bayliss 2782	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
	E. Cape, Elliott dist.	31° 31′ S, 28° 0′ E	Collector unknown																						
	E. Cape, King Williamstown dist.	32° 54′ S, 27° 23′ E	Batten s.n.																						
S. rexii × _S. gardenii_	Natal, Weza	30° 38′ S, 29° 35′ E	H & B 3486/3	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. primulifolius	Natal, Harding dist.	30° 34′ S, 29° 53′ E	Lennox s.n.	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
	Natal, Pietermaritzburg dist.	29° 35′ S, 30° 35′ E	Hilliard s.n.																						
	E. Cape, Port St Johns	31° 37′ S, 29° 33′ E	Hilliard 1042	·	×	×	×	·	×	×	×	×	·	·	·	·	·	×	·	·	·	·	·	·	·
	E. Cape, Bashee River Mouth	32° 15′ S, 28° 56′ E	J. Gordon-Gray s.n.	·	×	×	×	·	×	×	×	×	×	·	·	·	·	×	·	·	·	·	·	·	·
S. primulifolius Introgressed by _S. rexii_	E. Cape, East London	32° 52′ S, 28° 5′ E	H & B 3544	·	×	×	×	·	×	·	×	·	·	·	×	·	·	·	·	·	·	·	·	·	·
	E. Cape, Bashee River Valley	32° 10′ S, 28° 46′ E	R. Wood s.n.																						

TABLE 1 (*continued*)

POLYPHENOLIC COMPOUNDS SEPARATED BY TWO-DIMENSIONAL CHROMATOGRAPHY IN *STREPTOCARPUS* (*continued*)

SPECIES	LOCALITY		ACCESSION NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
S. primulifolius subsp. *formosus*	Natal, Uvongo River Gorge	30° 50′ S, 30° 23′ E	Strey 5786	·	×	×	×	·	×	×	·	·	×	·	·	·	·	×	·	·	·	·	·	·	·
	Natal, Oribi Gorge	30° 43′ S, 30° 14′ E	Hilliard 2791																						
	Natal, Rosslea, Umzinto dist.	30° 21′ S, 30° 22′ E	Hilliard 3122	·	×	×	×	·	×	×	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·
	Natal, Ellesmere, Umzinto dist.	30° 19′ S, 30° 25′ E	H & B 3813	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. cyaneus	Swaziland, Mbabane	26° 19′ S, 31° 8′ E	Akhurst s.n.	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
	Swaziland, Hlatikulu	26° 58′ S, 31° 19′ E	Ross 1762																						
	Transvaal, Sabie	25° 6′ S, 30° 48′ E	Hilliard 2959																						
	Transvaal, Havelock Border Post	25° 57′ S, 31° 6′ E	Hilliard 2950																						
S. parviflorus	Transvaal, Louis Trichardt	29° 55′ S, 23° 3′ E	Hilliard 4756	·	×	×	×	·	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
S. sp. aff. *parviflorus*	Transvaal, Graskop dist.	24° 56′ S, 30° 50′ E	H & B 6038	·	×	×	×	·	×	×	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·
S. gardenii	Natal, Richmond dist.	29° 52′ S, 30° 16′ E	H & B 5779	·	×	×	×	·	×	×	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
	Natal, Inanda dist.	29° 32′ S, 30° 45′ E	Strey 7547																						
	Natal, Weza	30° 38′ S, 29° 35′ E	H & B 5788	·	×	×	×	·	×	·	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. johannis	E. Cape, Lusikisiki dist.	31° 28′ S, 29° 38′ E	Hilliard 2101	·	×	×	×	·	×	·	×	×	·	·	×	×	·	·	·	·	·	·	·	·	·
	Natal, Weza	30° 38′ S, 29° 35′ E	H & B 5787	·	×	×	×	·	×	×	×	×	·	·	×	×	·	·	·	·	·	·	·	·	·
S. baudertii	E. Cape, Matatiele	30° 19′ S, 28° 48′ E	H & B 3778	·	×	×	×	·	×	×	·	×	·	·	·	·	·	·	·	·	·	·	·	·	·
	E. Cape, Bashee River Valley	32° 15′ S, 28° 56′ E	J. Gordon-Gray s.n.	·	×	×	×	·	×	×	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·
	E. Cape, East London	32° 56′ S, 28° 4′ E	H & B 3542	·	×	×	×	·	×	·	×	×	·	·	·	×	·	·	·	·	·	·	·	·	·
S. polyanthus	Natal, Pietermaritzburg	29° 35′ S, 30° 35′ E	Hilliard 1821	·	×	×	×	·	×	·	·	·	·	·	·	·	×	·	·	·	·	·	·	·	·
S. prolixus	Natal, Inanda dist.	29° 32′ S, 30° 45′ E	Strey s.n.	×	×	×	×	·	×	·	·	×	·	·	×	·	×	·	·	·	·	·	·	·	·
	Natal, Ndwedwe dist.	29° 34′ S, 30° 56′ E	Strey s.n.	×	×	×	×	·	×	·	·	×	·	·	×	·	·	·	×	×	×	·	·	·	·
S. prolixus × *S. polyanthus* subsp. *verecundus*	Natal, Inanda dist.	29° 32′ S, 30° 45′ E	H & B 3442	·	×	×	×	·	×	·	·	×	·	·	×	·	·	·	·	·	·	·	·	·	·
**S. pentherianus*	Natal, Paulpietersburg dist.	27° 25′ S, 30° 48′ E	H & B 6058	·	×	×	×	·	×	·	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·
SUBGENUS STREPTOCARPELLA																									
S. hilsenbergii	Madagascar		C 4619	·	×	×	×	·	·	·	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. glandulosissimus	*ex hort.* Hilliard		Source unknown	·	×	×	×	·	·	·	×	×	·	·	·	·	·	·	·	·	·	·	·	·	·
S. caulescens	Kenya		C. 1737	·	×	×	×	·	·	·	×	×	·	×	·	·	·	·	·	·	·	·	·	·	·
S. saxorum	Tanzania		C. 2908	·	×	×	×	×	·	·	×	×	·	·	×	·	·	·	·	·	·	·	·	·	·

*Denotes plants collected in the wild.
†H & B indicates Hilliard and Burtt.
Note: Where the phenolic compounds of a number of accessions are the same, the accessions are bracketed and the phenolic compounds are given only once in the Table.

In the present study the phenolic compounds in a number of taxa of *Streptocarpus* (Gesneriaceae) were investigated, to see whether they might throw any light on species limits and relationships in this taxonomically difficult genus.

Simon and Goodall (1968) have commented at some length on the use of two-dimensional chromatography and the problems encountered in chemotaxonomy. Greater resolution is to be expected from the use of a two-dimensional chromatographic technique. However, the identification of most, if not all, the observed compounds is beyond the scope of most biological investigations. However, according to Alston and Turner (1963) this limitation by no means eliminates the possibility of obtaining a great deal of useful systematic information. According to Simon and Goodall (1968) the number of compounds which can be detected is so much increased in comparison with one-dimensional chromatography, that this factor alone would allow for a better differentiation of taxa. Correct evaluation of the results is a difficult problem. Tabular listings are difficult to assess, particularly if the number of taxonomic units and compounds detected is large. The results of chemotaxonomic studies are generally evaluated on the basis of the presence or absence of compounds and for this reason, methods based on binary similarity indices have been applied (Simon & Goodall, 1968). Ellison, Alston and Turner (1962) proposed a method of expressing and visualizing quantitative relationships of species. These authors used the Jacquard similarity index, which they called the "paired affinity index" viz., $\frac{\text{Spots in common for species A and B}}{\text{Total spots in A and B}} \times 100$. They compared species of *Bahia* and related genera and expressed the quantitative relationships in the form of polygonal graphs. Harvey and Grant (1964) used a similar approach in a chemo-taxonomic study of the genus *Lotus* and they found that it was of more use in differentiating distantly related taxa, than closely related ones. Simon and Goodall (1968) used the same approach, but found that the similarity values were so high when species belonging to the genus *Medicago* were compared, that the pattern of species relationships was not readily discernible.

MATERIALS AND METHODS

The phenolic compounds were investigated in 55 accessions representing 27 species of *Streptocarpus*. Unless otherwise indicated in Table 1 the material used was from plants grown together under relatively uniform conditions in a shadehouse. Runemark (1968) maintains that only material cultivated under uniform environmental conditions is suitable for use in chemotaxonomic studies. However, in this study it was found that, within a species, the phenolic patterns of plants from the wild and plants under cultivation corresponded.

Plant material was collected during the summer of 1968/69 and chromatographed within three months of collection. Leaves from each accession were ovendried at 55—60° for 48 hours. The material was then finely ground and stored in stoppered containers at 0°. The plant material was extracted using the technique of Bate-Smith (1956; 1962). 10 ml 2N HCl was added to a test tube containing approximately 0 ·5 gm plant material. This was then heated for 20 min in a boiling waterbath. The extract was then cooled, filtered and transferred to a smaller test tube and 0 ·5 ml iso-amyl alcohol was added. The mixture was shaken vigorously and then allowed to stand for the different layers to separate out. A 50μl aliquot of the hydrolysate from each accession was spotted on each of two sheets of Whatman No. 1 chromatography paper (28 cm × 23 cm).

TABLE 2

Compounds isolated from two-dimensional chromatograms, their R_f values and recorded colours

Compounds	R_f in:		Colours observed				Identification
	BAW	Forestal	Visible	Ultra-violet	*p*-Nitro-aniline	Benedict's	
1	0·53	0·46	purple	purple	—	—	Cyanidin
2	0·56	0·80	—	blue	light brown	yellow	
3	0·81	0·85	—	blue	light brown	yellow	
4	0·91	0·72	—	blue	—	—	
5	0·41	0·56	—	—	—	yellow	
6	0·93	0·76	—	—	—	green-yellow	
7	0·98	0·63	—	—	—	orange	
8	0·28	0·61	—	—	—	yellow	
9	0·46	0·72	—	—	—	yellow	
10	0·74	0·87	—	—	brown	brown	
11	0·88	0·86	—	blue	—	yellow	
12	0·88	0·57	—	—	—	yellow	
13	0·63	0·85	—	purple	brown	bright yellow	
14	0·76	0·84	yellow	blue	pink	—	
15	0·62	0·82	—	—	—	light pink	
16	0·24	0·65	purple	—	—	—	
17	0·71	0·71	—	—	brown	—	
18	0·17	0·58	—	—	—	yellow	
19	0·98	0·89	orange	purple	orange	orange	Dunnione
20	0·97	0·62	—	—	—	purple	
21	0·34	0·27	—	blue	—	—	
22	0·45	0·79	—	purple	—	yellow	

These were developed using the ascending technique in the following solvents: (a) first phase, in the machine direction of the paper, the organic phase of BAW (butanol—acetic acid—water 4:1:5 v/v); (b) second phase, Forestal solvent (acetic acid—hydrochloric acid—water 30:3:10 v/v). (The papers were equilibrated for 18 hours with the lower phase of the BAW solvent.) After drying, the chromatograms were examined in visible and UV-light. (Hanovia Chromatolite, 2537Å). One replicate was sprayed with diazotized *p*-nitro-aniline reagent (Swain, 1953) and the other with Benedict's reagent prepared according to Hänsel (1959).

Results and Discussion

Preliminary work in this investigation was done using a one-dimensional chromatographic technique. However, insufficient resolution was obtained and these results have been omitted. A two-dimensional technique was then used because of the greater resolution to be expected.

However, it was still found that relatively few phenolic compounds were separated out. Their positions on the chromatogram are given in Fig. 1. Their R_f values and recorded colours are given in Table 2. If one compares the 66 compounds separated out by Ellison, Alston and Turner (1962) from leaves of species of *Bahia* with the 22 separated out from the leaves of species of *Streptocarpus* in this study, it becomes obvious that the latter must be regarded as being very poor in phenolic compounds. Evaluation of the results, which are given in Table 1, was thus very difficult and no clear-cut answers to taxonomic problems in the genus emerged.

Compound 1 was identified as cyanidin. This pigment gave a purple colour to the abaxial surface of leaves of *S. wendlandii*, *S. molweniensis* and *S. prolixus*. Although it was only found in these three species in the present study, its presence has been reported in numerous species of *Streptocarpus* (Lawrence & Sturgess, 1957). Cyanidin often appears in plants growing under relatively unfavourable conditions. Because of its wide occurrence it is of no value as an indicator of species limits.

Compound 17 is also a pigment and its occurrence seems to be related to the occurrence of cyanidin. These two compounds, together with dunnione (compound 19) were the only ones which could be distinguished in daylight before the chromatograms were sprayed with a reagent (Table 2). All the other compounds were visible only under ultra violet light or after spraying with one of the reagents.

Compound 4 was identified as coumaric acid and the identification was confirmed using one-dimensional chromatography and comparing the hydrolysates with authentic compounds (Koch Light, England) which were chromatographed under the same conditions.

The results were analysed using the "paired affinity index" and polygonal representation as used by Ellison, Alston and Turner (1962). These results have not been presented as it was found that the similarity values were so high that no pattern of species relationships could be discerned. Nevertheless, a number of interesting facts emerged. Perhaps the most interesting of these is that while compounds 2, 3, 4 were present in all the species studied (27 out of a total of about 145 in the whole genus), compound 6, common to all the true *Streptocarpus*, was absent from the species belonging to the sub-genus *Streptocarpella* (4 studied out of a total of about 40).

A number of species showed a common pattern of phenolic compounds; viz. 2, 3, 4, 6, 7, 8, 9. These were the three accessions of *S. rexii* from E. Cape; two accessions of *S. primulifolius* from Harding dist. and Pietermaritzburg dist., Natal; *S. primulifolius* subsp. *formosus* from Ellesmere, Umzinto dist., Natal; the two accessions of *S. cyaneus* from Swaziland and the two from the Transvaal; a hybrid *S. rexii* ×*S. gardenii* from Weza, Natal; and the accessions of *S. gardenii* from Inanda dist. and Richmond dist., Natal. The fact that *S. rexii*, *S. primulifolius*, and *S. cyaneus* have a common phenolic pattern is, perhaps, not surprising as these very closely related taxa could well be regarded in the broad sense as being one species (Hilliard & Burtt, 1969). *S. gardenii*, taxonomically close to *S. rexii* and its allies, is also very closely related chemically.

There is considerable variation in the phenolic patterns shown by the different accessions of *S. primulifolius* subsp. *formosus*. These accessions show some morphological variation and this is apparently paralleled in their phenolic patterns. The accessions of *S. primulifolius* show three different phenolic patterns which appear to be related to their geographical distribution. The two accessions from Natal have compounds 2, 3, 4, 6, 7, 8, 9; the accession from Bashee River (The Haven), E. Cape has compounds, 2, 3, 4, 6, 7, 8, 9, 10, 15 and the accession from Port St. Johns (situated between the other two localities) has compounds 2, 3, 4, 6, 7, 8, 9, 15

Although *S. candidus* is taxonomically distinct from the *S. rexii*, *S. cyaneus* and *S. primulifolius* group, it appears to be closely related chemically and shows the same phenolic pattern as *S. primulifolius* from Port St. Johns, E. Cape.

A similar phenolic pattern is shown by *S. primulifolius* subsp. *formosus* (Natal, Rosslea, Umzinto dist.), *S.* sp. aff. *parviflorus* (Transvaal, Graskop) and *S. baudertii*, (E. Cape, Bashee River Valley). The chemical similarity of the first two is understandable on taxonomic grounds: *S. parviflorus* is closely related to *S. cyaneus* and thus to *S. primulifolius* and *S. rexii*, but *S. baudertii* is morphologically very different (Hilliard & Burtt, 1969).

S. pole-evansii and *S. dunii* were the only accessions to have compound 19 which is known to be the pigment dunnione. This is in agreement with the

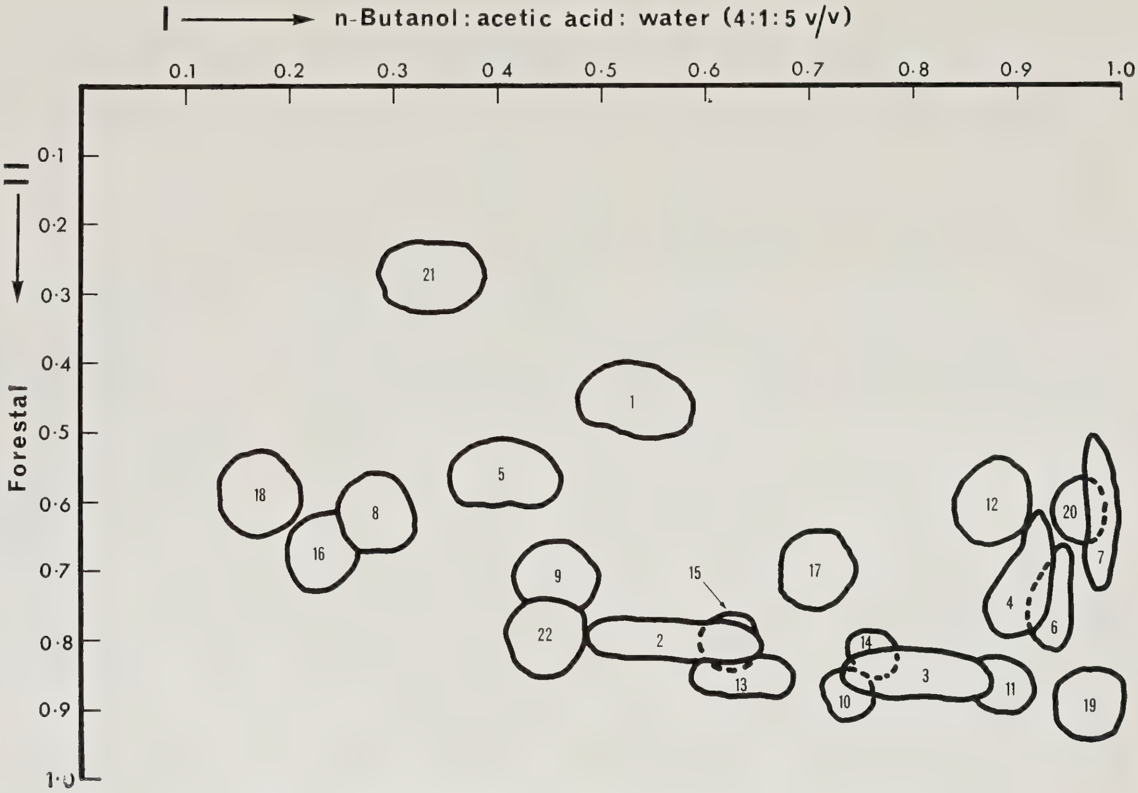

FIG. 1. Master chromatogram showing the position of each of the 22 compounds observed.

findings of Harborne (1966). The pigment is extruded as red granules over much of the epidermal layer.

S. kentaniensis and *S. trabeculatus*, both distinctive and taxonomically isolated species, have unique phenolic patterns. This appears to be true of *S. grandis* too, but none of its close allies have been investigated. This serves to emphasize that the narrowness of the spectrum of species studied is also a limiting factor in interpreting the results.

Any future work on the phenolics of *Streptocarpus* should be done so as to include a far wider range of species than was done in this preliminary survey. Interesting work also remains to be done to compare the phenolic patterns obtained from flowers with those obtained from leaves.

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